Kidney International, Vol. 35 (1989), pp. 1073–1086

NEPHROLOGY FORUM

The role of adaptation in allograft acceptance

Principal discussant: ROBERT A. P. KOENE

University of Nijmegen, The Netherlands

Case presentation

A 40-year-old male was admitted to the University Hospital of Nijmegen because of rapidly progressive renal insufficiency. Two weeks before admission, he had been given penicillin injections because of a sore throat and fever. Three days later he developed macroscopic hematuria, arthralgias, and hemoptysis. On admission, his blood pressure was normal. There was no presacral or peripheral edema. Physical examination of the heart and lungs revealed no abnormalities. There was no skin rash. The remainder of his general examination was unremarkable. Urinalysis revealed 1+ protein, no glucose, many red blood cells, and occasional red blood cell casts. Urine output was 400 ml in the first 24 hours. The serum creatinine was 11.1 mg/dl; endogenous creatinine clearance, 1.5 ml/min; BUN, 88 mg/dl; and 24-hour protein excretion, 1.0 g. The serum C3 was normal. Anti-GBM antibodies were not present in the serum. Antibodies against neutrophil cytoplasm (ANCA) were not measured. The chest x-ray disclosed bilateral pulmonary infiltrates. A percutaneous renal biopsy disclosed diffuse, necrotizing, extracapillary glomerulonephritis with granular staining of IgM and C3 along the glomerular capillary wall. Small vessels were normal; larger vessels were not present in the biopsy specimen. A diagnosis of systemic necrotizing vasculitis was made, and the patient was given prednisone, 60 mg, and cyclophosphamide, 150 mg, daily. Hemodialysis was started, but subsequently his renal function improved, and the pulmonary lesions disappeared; one month after admission, hemodialysis was discontinued. On discharge, creatinine clearance was 19 ml/min. Despite continuous treatment with cyclophosphamide (100 mg/day) and low-dose prednisone (10 mg/day), his renal function gradually deteriorated. After one year, hemodialysis again was necessary.

At age 42, after 12 months of hemodialysis, the patient received a cadaveric renal allograft. The crossmatch was negative and there was one HL-A incompatibility at the B locus; HL-A–DR typing was not carried out. The right donor kidney had two arteries that were anastomosed separately to the left external iliac artery. Conventional immunosuppressive treatment (azathioprine, 150 mg/day) and prednisone (initial dose, 200 mg/day; maintenance dose, 15 mg/day) was employed. The allograft functioned immediately. The patient was discharged on the 26th postoperative day. At that time, blood pressure was 140/80 mm Hg, creatinine clearance was 73 ml/min, and an intravenous urogram showed no abnormalities.

After 2 months, the patient developed hypertension (170/110 mm Hg) and proteinuria of 1–2 g/liter. Allograft rejection was diagnosed after 6 months on the basis of increased proteinuria and a gradual rise of the serum creatinine. Treatment with a short course of high-dose oral prednisone did not improve renal function. Renal biopsy, performed one year after transplantation, revealed chronic vascular rejection. Because the hypertension was severe and responded only partially to treatment with propranolol, minoxidil, and furosemide, renal arteriography was performed 17 months after transplantation (Fig. 1). The arteriogram disclosed 90% stenosis (with poststenotic dilation) of the origin of the artery supplying the lower pole of the graft. Appearance of the contrast medium was delayed in the lower pole. The upper artery, which supplied the major part of the kidney, was slightly narrowed at its origin, but filling of its branches was not delayed. Because the contribution of the lower renal artery stenosis to the severe hypertension was not clear, and because the biopsy showed severe vascular rejection, reconstruction of the stenosis was not attempted. Renal function gradually deteriorated further. Six months later, almost 2 years after transplantation, hemodialysis was resumed and the allograft was removed.

The grafted kidney weighed 110 g; a surprising difference existed between the lower pole (supplied by the stenotic artery), which had a depressed, smooth, and brown-red surface, and the remaining four-fifths of the kidney, which had a pale, swollen aspect and a fine nodular surface (Fig. 2). On longitudinal section the lower pole appeared to be atrophic. Here the cortex was narrow and sharply demarcated from the medulla. The remainder of the graft had a cortex of normal width with a brownish-yellow color and a poor demarcation from the medulla. Histologic examination revealed chronic vascular rejection in the upper part of the kidney, findings consistent with those in the previous renal biopsy. Focal and segmental proliferation of mesangial cells was present in the glomeruli, as were thickening and splitting of the basement membrane. The glomerular capillaries showed focal adhesions to Bowman's capsule, and a few epithelial crescents were visible (Fig. 3A). The tubules were atrophic and interstitial fibrosis was present. The arterial vessels showed extensive intimal fibrosis. Immunofluorescent microscopy revealed glomerular deposits of IgM, C1q, C4, and C3, predominantly in the capillary walls (Fig. 3B). By contrast, the lower pole (supplied by the stenotic artery) showed only signs of chronic ischemia. The otherwise-normal glomerular capillaries were slightly collapsed and the tubules were markedly atrophic; the glomeruli thus appeared crowded (Fig. 3C). No immunoglobulin deposits were found in the glomeruli or in the vessels (Fig. 3D).

After removal of the transplanted kidney, cytotoxic antibodies of broad specificity, predominantly directed against B lymphocytes, were

Presentation of this Forum is made possible by an educational grant from Bayer AG/Miles Inc. This Forum was presented in Talloires, France, in May 1988.

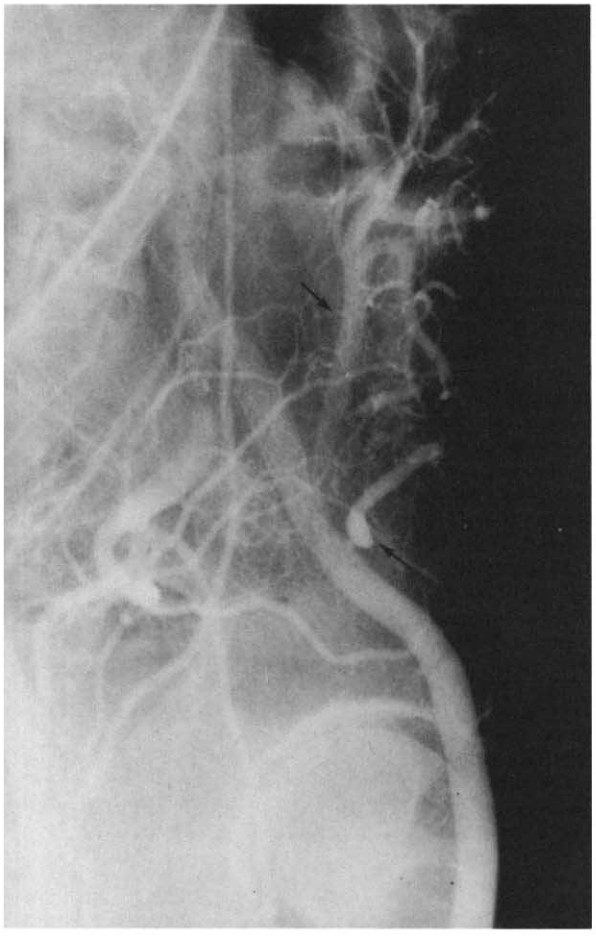

Fig. 1. *Angiogram of renal allograft.* The two renal arteries (*arrows*) have been anastomosed separately to the left external iliac artery. Contrast medium already has reached the upper part of the kidney via the normal upper artery. The lower artery shows a severe stenosis at its origin with a poststenotic dilation and delayed appearance of contrast medium.

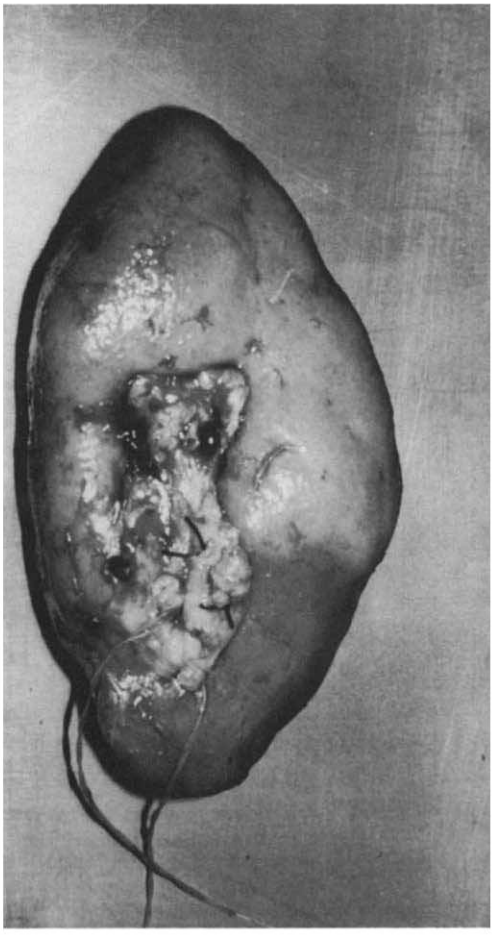

Fig. 2. *Macroscopic appearance of kidney graft after removal.* The upper part of the kidney is pale and swollen. The lower pole is atrophic.

demonstrable in the serum during a period of 3 months. Thereafter, these antibodies disappeared. Four months after transplantectomy, he received a second transplant. After seven months this second kidney was lost as a consequence of a vascular rejection.

Discussion

PROF. ROBERT A. P. KOENE (*Professor of Nephrology, Nijmegen University School of Medicine; Head, Division of Nephrology, University Hospital, Nijmegen, The Netherlands*): This patient is unusual in that he had two renal arteries in his transplant kidney; significant stenosis developed in the lower artery, and the "protected" and "unprotected" parts of the transplanted kidney responded differently from each other. The post-stenotic segment of the transplanted kidney in this patient apparently was protected against immune attack by the host. The ongoing rejection process that caused loss of the graft probably was governed by destructive antibodies. In fact, lymphocytotoxic antibodies were demonstrable in the circulation of the patient during the first weeks after the transplanted kidney was removed. In the normally perfused upper segment of the graft, pathologic examination revealed a typical picture of so-called transplant glomerulopathy with extensive deposition of immunoglobulins and complement components in the capillary walls. The critical observation, the apparent protection of the lower pole, has many features in common with a phenomenon that has been observed in experimental transplantation of organs and tissues, that is, the occurrence of "graft adaptation." This phenomenon will be the central focus of my discussion. The findings in this patient resemble reports on a few patients with unilateral glomerulonephritis in whom the unaffected kidney showed signs of decreased blood flow as a consequence of renal artery stenosis or long-standing hydronephrosis. I will discuss these experimental and clinical observations, delineating the factors that caused the exceptional form of partial graft protection in this patient.

Factors in long-term graft acceptance

How can we best assure our patients of the long-term success of renal transplantation? The ideal method of obtaining long-term graft survival is complete matching of donor and recipient histocompatibility antigens. In clinical transplantation, unfortunately, this can be achieved only in identical twins. Nevertheless, long-term graft survival can be achieved in histoincompatible combinations, and it is well known that with increasing time after transplantation, the risk of rejection diminishes. Table 1

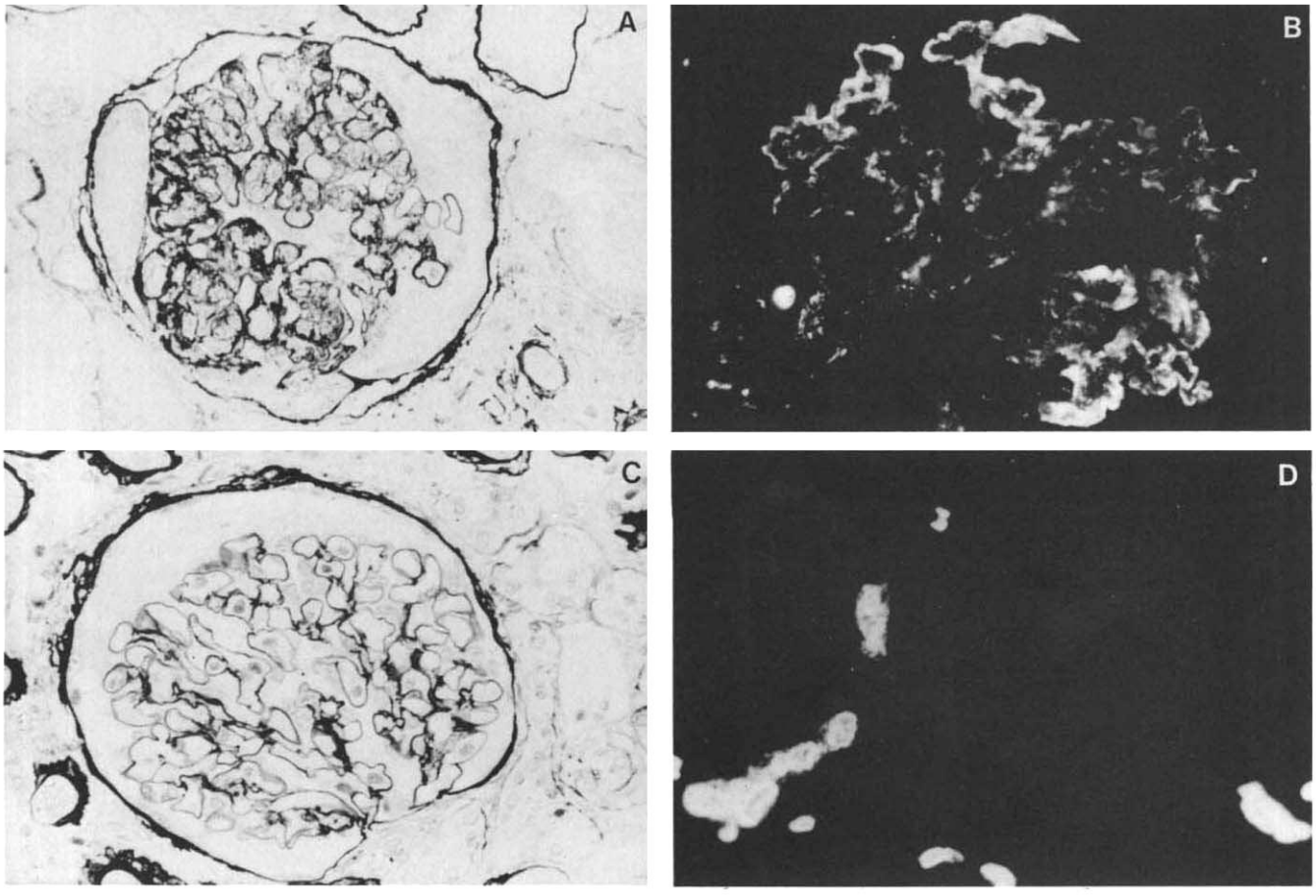

Fig. 3. *Histology of removed graft.* A glomerulus in the upper part of the kidney shows "transplant glomerulopathy" (**A**) with deposits of IgM predominantly along the capillary wall (**B**). A glomerulus in the lower, poststenotic segment shows only signs of ischemia (**C**). In this part IgM is present in tubular casts, but not in the glomerular capillaries (**D**).

lists factors that play a role in long-term graft survival. In the host, nonspecific suppression of the immune reaction by corticosteroids, antimitotic drugs, or cyclosporine is important. Other, more specific, protective mechanisms also might be operative, such as deletion of antigen-reactive cells (the definition of tolerance), preferential proliferation of suppressor T-cells, or the production of specific, blocking (so-called enhancing) or antiidiotypic antibodies. Although the existence of these specific protective mechanisms can be demonstrated in selected animal models, the evidence for their presence in human transplantation is weak. Lymphocytes of patients with long-established, well-functioning kidney grafts no longer generate an in-vitro, cytotoxic response to donor cells. Their response to cells from individuals of different HL-A type remains normal [1]. Whether specific unresponsiveness is due to the generation of suppressor T-cells or to elimination of antigen-reactive clones is not known. Some hemodialysis patients do develop blocking antibodies after multiple blood transfusions; these antibodies are directed against the receptors of the T-cells specifically involved in the recognition of a particular HL-A antigen (antiidiotypic antibodies). Kidneys of the right HL-A specificity subsequently transplanted into these patients survive for prolonged periods [2]. These interesting data, however, do not help to clarify the striking observations in the case presented, because changes in the graft and not in the host probably were responsible for the protection of the lower part of the graft.

Table 1. Factors contributing to long-term graft survival

Host
Nonspecific immunosuppression
Deletion of antigen-reactive cells (tolerance)
Suppressor T-cells
Antibodies (enhancing or antiidiotypic)
Graft
Loss of passenger cells
Adaptation

Where do graft factors influence the outcome of transplantation? Changes in the graft per se can affect the immune response at two distinct levels (Fig. 4). At the afferent level, the concept of the so-called passenger cell has received much attention. In addition to resident cells, most organs and tissues harbor circulating and semisessile cells derived from the bone-marrow, such as lymphocytes, monocytes, macrophages, and dendritic cells. During the first weeks after transplantation, these cells gradually disappear from the graft and are replaced by host cells, hence their name "passenger cells." These passenger

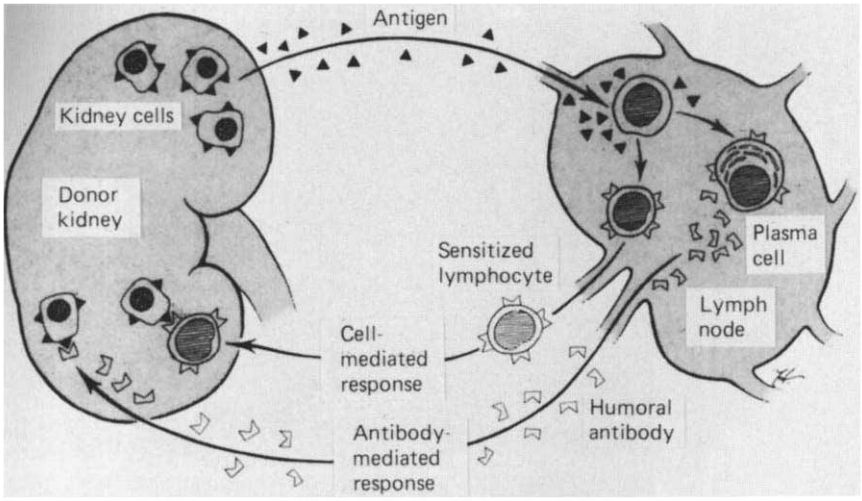

Fig. 4. *Schematic representation of the allograft reaction.* Foreign antigens (either in soluble form or on the surface of passenger cells) are released from the graft and recognized in the draining lymph nodes (*afferent limb*). This leads to the formation of sensitized lymphocytes and antibodies that attack the graft (*efferent limb*). (From Ref. 3.)

cells, especially the dendritic cells and monocyte-macrophages, play an important role in antigen presentation and thus in the triggering of the immune response. Their role has been demonstrated most convincingly in experimental transplantation of thryoid and pancreatic tissue. Passenger cells can be removed from these tissues by a short period of culture prior to transplantation; subsequent grafting leads to prolonged and often lifelong survival [4, 5]. The observation that the grafts were promptly rejected when the recipients were challenged with donor lymphoid cells [6, 7] demonstrated that the prolonged survival indeed was caused by a loss of the passenger cells, and not by a loss of antigenicity during culture. This mechanism could be important for transplantation in the future, especially for pancreatic transplantation. I will not discuss it further here, however, because it concerns a decrease in immunogenicity of a tissue to be transplanted into a host who has not encountered the foreign graft antigens earlier. In contrast, the phenomenon of protection of part of the kidney observed in today's patient occurred in the presence of an ongoing immune response.

Definition of graft adaptation

The first description of graft adaptation stems from the work of the Woodruffs carried out almost 40 years ago [8]. They grafted allogeneic thyroid tissue to the anterior chambers of the eyes of guinea pigs. The grafts became vascularized from the host iris yet showed permanent survival in most of the recipients. If thyroid tissue from the same donor was grafted subcutaneously simultaneously with the eye grafts, both thyroid grafts were rejected. If subcutaneous grafts were placed 5 to 6 weeks after the anterior chamber transplants, however, the established grafts in the eye underwent no changes, but the subcutaneous grafts were rejected. From these observations Woodruff hypothesized that ". . . homo-transplants become less vulnerable as time goes on, and, after a certain critical period, are capable of surviving in the face of a high degree of immunity in the recipient, which they would not have been able to withstand earlier in their life history" [9]. In later studies Woodruff observed a similar phenomenon in skin grafts in rats. Second skin grafts applied to recipients already carrying a well-established skin graft from the same donor sometimes were rejected while the first graft remained viable [10]. Woodruff coined the term "adaptation" in his first study, and this term has been used by subsequent investigators for this puzzling phenomenon. Some authors have used "adaptation" to designate decreased immunogenicity of the graft by the removal of passenger cells, but this phenomenon utilizes a completely different mechanism of graft protection, so I prefer to use to the classic definition coined by Woodruff. I use the term "adaptation" only for situations in which the graft is protected against an intact or ongoing immune response.

Now I will explore the different aspects of adaptation against cell-mediated and antibody-mediated rejection and then turn to the mechanism of adaptation. I will close with a discussion of the important role of hemodynamic factors.

Adaptation to cell-mediated rejection

In the few studies of the phenomenon of adaptation that followed the original observations by Woodruff, murine skin grafts have been used as the primary model. When allogeneic skin is grafted onto untreated mice, it usually is rejected between 10 and 15 days after transplantation. Graft survival can be prolonged by immunosuppressive treatment, however. By treating the recipient mice with antilymphocyte serum during the first 5 days after transplantation, Nirmul et al prolonged graft survival to 30 days [11]. When a second graft from the same donor was placed 14 days after the first graft, this second graft was rejected after 10 to 12 days, when the first graft showed no signs of rejection. We obtained comparable results using a different immunosuppressive regimen consisting of specific, blocking (so-called enhancing) antibodies, combined with whole-body irradiation on the day prior to grafting [12]. Mice carrying a skin allograft received a second graft of the same donor strain after 13 days. The pattern of rejection of the first and second grafts is shown in Figure 5. We found a significant 2- to 4-day difference in survival between the two grafts. The first grafts showed no visual evidence of destruction when the second grafts were totally destroyed; the first grafts thus met the criterion for graft adaptation. Histologic studies of skin grafts in these models have shown that the rejection process is cell mediated. The findings I have described therefore prove that adaptation to cellular rejection occurs. The adaptation is not caused by a decreased immune responsiveness

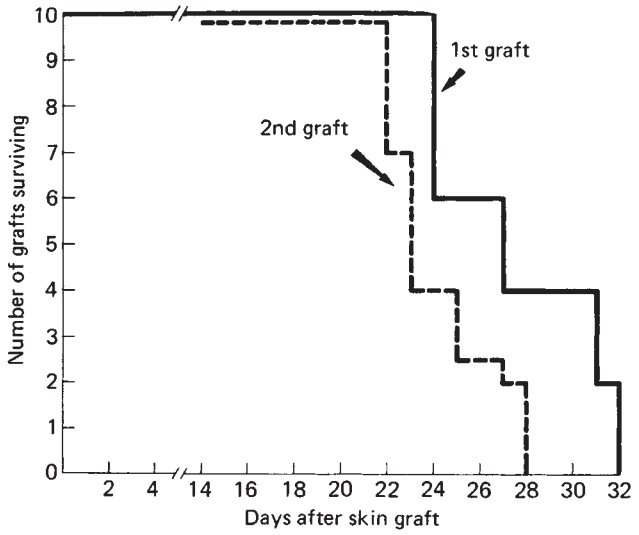

Fig. 5. *Adaptation to cell-mediated rejection.* Allogeneic skin was grafted onto immunosuppressed mice. The first grafts survived for 27.7 + 2.0 days, whereas second grafts from the same donor strain, placed on day 13, survived only until 23.8 + 1.8 days. (From Ref. 12.)

of the host, because only the first grafts were protected, whereas the rejection process was ongoing in the second grafts. The changes and mechanisms responsible for this protection must be sought in the graft itself.

Removal of a longstanding graft from its original recipient and retransplantation to a new, untreated animal seems an obvious way to study the mechanism of allograft protection. Nirmul et al regrafted 7-day-old skin grafts, but the transferred grafts did not show prolonged survival as compared with non-transferred first grafts [11]. Retransplantation experiments also have been performed with long-surviving renal allografts in rats. In selected combinations of inbred strains of this animal, it is relatively easy to induce life-long graft survival with simple immunosuppressive regimens, such as a few injections of enhancing antibodies or antilymphocyte serum. When renal allografts that had survived for more than 6 months were retransplanted to otherwise normal, untreated rats of the same inbred strain as the original recipient, the onset of rejection was delayed in comparison to that in control animals [13]. Similar results were obtained in another study, in which retransplanted kidneys survived 3 to 6 times longer than did fresh grafts placed in control animals [14]. One should be careful, however, to attribute these results to adaptation as earlier defined. In these retransplantation experiments, changes in the afferent arm of the response—that is, decreased immunogenicity of the graft—cannot be distinguished from a decreased sensitivity to the rejection mechanisms in the efferent arm. The prolonged survival thus also can be explained by the disappearance of passenger cells from the graft during their prolonged residence in the primary host. Loss of passenger cells does indeed seem to be the most likely explanation for the prolonged survival. Subsequent studies have demonstrated that the intravenous transfer of small amounts of dendritic cells of the original donor to the recipient at the time of retransplantation abolishes the difference in survival between retransplanted and primary transplanted kidneys [15]. A study of the mechanism of adaptation therefore requires examination of models in which only changes in the efferent arm of the immune response occur.

Adaptation to antibody-mediated rejection

Models that measure the resistance of fresh and long-term grafts to the destructive effects of specific antibody have important advantages for the study of the mechanism of graft adaptation. Antibodies to major histocompatibility complex (MHC) antigens can be administered experimentally to recipients in standardized reproducible doses; these antibodies are cytotoxic and act only in the effector arm of the immune response. Again, skin grafting in the mouse is an attractive model, because the technique is simple and the destruction, or rejection process, can be followed visually. Skin grafts in the mouse, however, are not very susceptible to the destructive action of cytotoxic antibodies, probably because the murine complement system is relatively inefficient [16]. This problem can be overcome either by administration of the antibody in combination with an effective heterologous complement source, such as rabbit complement, or by choosing donor skin grafts of great antigenic disparity, such as xenografts of rat skin, instead of allografts [17]. The basic model used in our studies is as follows: Allogeneic or xenogeneic skin is placed onto immunosuppressed recipients. Immunosuppressive treatment is aimed at maximal elimination of the immune response so that rejection is prevented and the grafts will show prolonged, preferably life-long, survival. An alternative and even more attractive model used by us is the nu/nu mouse; these mice are athymic, virtually lack T-cells, and consequently do not reject most foreign grafts. Thus, well-established healthy grafts can be obtained. The recipients are then challenged by the intravenous or intraperitoneal injection of antiserum that contains antibodies directed against the graft antigens. If the antigenic difference is small, rabbit complement is given in addition. Sensitivity of the graft to this treatment is manifested by macroscopic and microscopic signs of necrosis, mostly within 24 to 48 hours after the injection [18]. Figure 6 shows how the sensitivity of skin grafts is modified by a prolonged residence on the recipient. In this particular experiment, B10.LP nu/nu mice received skin grafts from histoincompatible B10.D2 mice. The B10.LP nu/nu recipients then received an injection of anti-B10.D2 serum, together with rabbit complement. Injections of antiserum given during the first 3 days after transplantation were ineffective, consistent with earlier observations showing that a period of 4 to 5 days after transplantation is required for adequate vascularization of the graft [19]. After this period, there is a rapid rise in sensitivity of the grafts, which is maximal around day 8. Injections given later gradually become less effective. The decreased sensitivity is not only reflected in a diminishing percentage of partially and totally necrotic grafts, but also in the time necessary for complete necrosis to occur after the injection of antibodies and complement, that is, from 24 to 48 *hours* at day 8, to 5 to 7 *days* with injections given at day 28.

To prove that the decreased sensitivity at days 14 to 50 was indeed caused by changes in the graft and was not influenced by decreased immune responsiveness of the host, we induced antibody-mediated rejection in a single recipient carrying two grafts, which were transplanted at different times. The results of the experiment are depicted in the upper panel of Figure 7. A

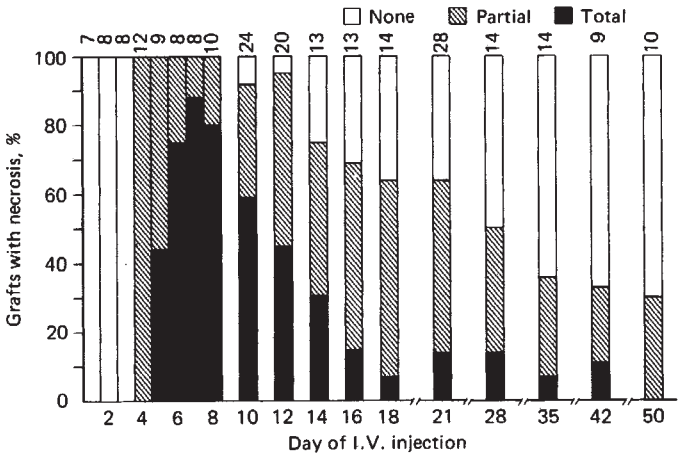

Fig. 6. *Adaptation to antibody-mediated rejection.* Effects of a single injection of alloantiserum and rabbit complement on skin allografts of increasing age in nu/nu recipients. Numbers on top of the diagram refer to the total number of animals used in each experimental group. (From Ref. 18.)

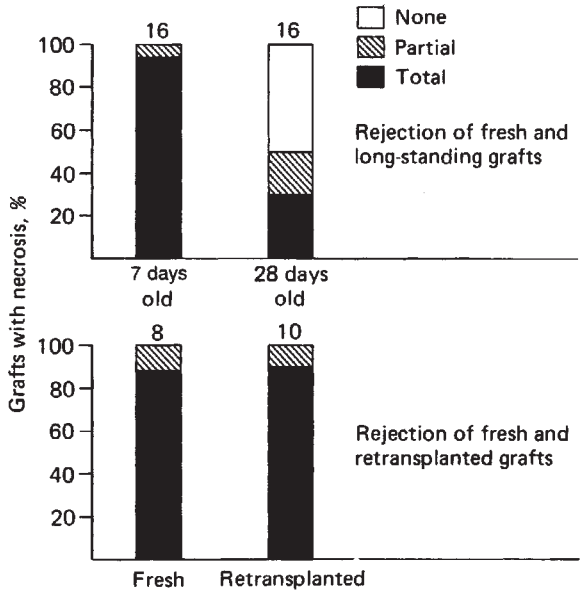

Fig. 7. *Mechanism of adaptation.* A second skin allograft transplanted 21 days after the first is completely sensitive to antibody and rabbit complement, whereas the older grafts placed on the same recipient show adaptation (*upper panel*). Retransplantation of 28-day-old grafts to new recipients re-establishes the sensitivity of these grafts to that of fresh (7-day-old) grafts (*lower panel*). (Modified from Ref. 18.)

second B10.D2 skin graft was transplanted onto the B10.LP nu/nu recipients 21 days after the first graft; antibody and complement were injected 7 days later. The older grafts showed decreased sensitivity to the antibody and complement, whereas the new grafts were rapidly destroyed. Thus decreased efficacy of the immunologic effector mechanisms of the recipients was eliminated as an explanation for the protection of the older grafts. The phenomenon meets the definition of adaptation; the older grafts were protected against an intact immune reaction, which was artificially induced by the administration of antibody in this model. Adaptation therefore is a general phenomenon occurring not only in cellular but also in antibody-mediated immune reactions.

Mechanisms of adaptation

What accounts for this series of seemingly disparate observations? Several hypotheses seek to explain the adaptation phenomenon. One is the assumption that during long-term residence of the graft, the endothelium of the graft vessels is gradually replaced by the host endothelium, and that this process leads to the disappearance of foreign antigens. Because the graft endothelium is considered the primary target for both cell-mediated and antibody-mediated rejection [20], adaptation to both processes can be expected in spite of the continued presence of donor antigen in the tissues beyond the vascular borderline and in spite of an intact immune-response system. A second hypothesis is that changes in the expression of the MHC antigens in the graft decrease the ability of the effector systems to recognize the graft as foreign. Yet a third hypothesis is that changes in vascularization, and thus perfusion, of the graft can occur, and that these alterations can decrease the amount of cellular or humoral mediators delivered to the graft. The balance between destructive and repair mechanisms may be altered to the advantage of the latter, and the graft can escape from the destructive process. Let us explore these three hypotheses.

Replacement of endothelium. Vascularization of full-thickness skin grafts, which occurs via the formation of anastomoses between recipient and donor vessels, leads to an established blood flow in the graft after 4 to 5 days. If donor endothelium were subsequently replaced by that of the host, insensitivity of the established graft to injected antibody would continue to exist even after retransplantation of the graft to a new host. Figure 7 (lower panel) shows that this is not the case for murine skin allografts, however. Grafts that had survived for 28 days on their primary hosts regained complete sensitivity to antibody after retransplantation. Much disagreement exists in the literature about this issue; some investigators have found evidence for endothelial replacement in rat skin grafted to mice [21], but we have not been able to confirm this finding. Even 170 days after transplantation, we find persistent expression of donor antigens on the vascular endothelium and are able to induce antibody-mediated destruction [22]. Nor has evidence been obtained for endothelial replacement in primarily vascularized grafts, such as kidney or heart grafts [23, 24]. Replacement of endothelium therefore does not seem a satisfactory explanation for graft adaptation.

Decreased MHC antigen expression. The glycoproteins that form the structural basis of the MHC antigens can be divided into two classes. Class-I antigens consist of two chains: a 45,000 dalton heavy chain penetrating the cell membrane is non-covalently linked to a 12,000 dalton chain known as β-2-microglobulin. The genes coding for the heavy chains are polymorphic; in humans they are located on the HL-A–A, -B, and -C loci of the MHC on the sixth chromosome; β-2-microglobulin is coded for outside the MHC on the 15th chromosome. Class-II antigens consist of two almost equally large chains of 34,000 and 29,000 daltons. These also are non-covalently linked and both are anchored in the cell membrane. In humans, class-II antigens are coded for by polymorphic genes in the D region of the MHC. Antigenic structures

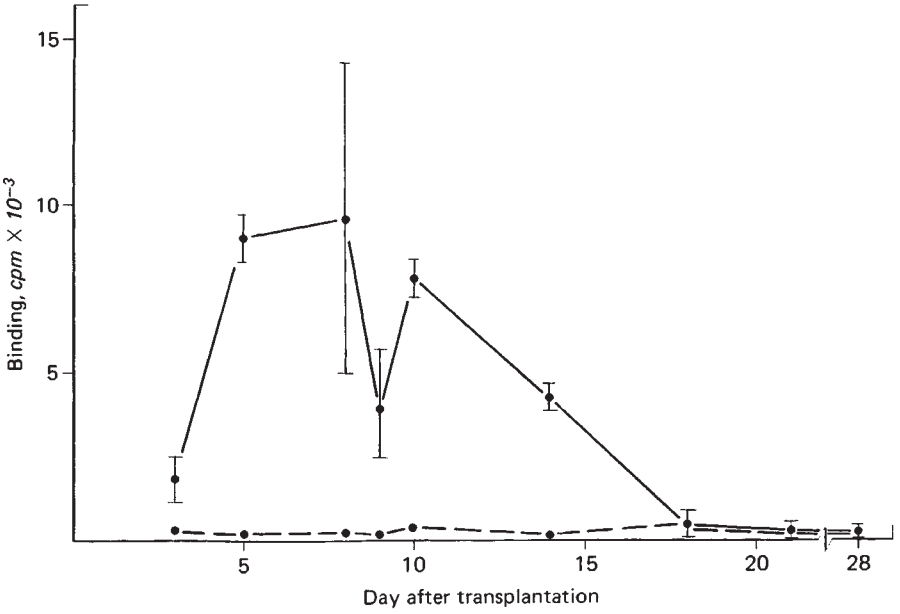

Fig. 8. *In-vivo binding of radiolabeled anti-class-I monoclonal antibody to mouse skin allografts.* The recipients were treated with cyclosporine to prevent rejection. Injected radioactivity was 6×10^4 cpm per mouse. Grafts were removed after 4 hours, and radioactivity of the entire graft was counted (●——●). MHC-compatible skin grafts carried by the same recipients were used as controls (●---●). (From Ref. 35.)

closely similar to those in humans have been detected in many species but have been studied most extensively in the mouse; in this species the class-I and -II antigens have been termed H-2 and Ia antigens respectively.

The tissue distribution of the two classes of MHC antigens is quite different. Class-I antigens are present on virtually all cells, but when the immune system is not noticeably stimulated, differences exist in the levels of antigen expressed (reviewed in Ref. 25). Under basal circumstances, the expression is mostly low or even undetectable on neuronal cells, striated muscle cells, exocrine gland cells, corneal endothelium, trophoblast, and mature spermatozoa. Class-II antigens are primarily expressed on the bone-marrow-derived cells that have a function in the immune response (cells of the monocyte-macrophage lineage, dendritic cells, Langerhans cells, B-cells, and stimulated T-cells). Class-II antigens also can be found, generally sparsely distributed, on some other cell types. In humans, endothelial cells express class-II antigens, and these antigens also are weak and patchily expressed on renal proximal tubular cells.

The variability of MHC antigen expression was first noted in cell cultures in vitro. Soluble mediators released by activated T-cells stimulate the expression of MHC antigens. The soluble mediators not only increased the concentration of MHC antigens, but also could induce the expression of class-II antigens on non-lymphoid cells that were previously negative (reviewed in Ref. 25). The interferons, and especially interferon-γ, proved to be the most potent stimulators of MHC antigen expression [26]. The factors causing increased expression of MHC antigens in vivo probably are similar to those identified in vitro. Activation of T-cells during allograft rejection and graft-versus-host disease leads to considerable increases of MHC antigen expression. Non-allogeneic stimuli, such as contact hypersensitivity, infection, or autoimmune disease, also can increase MHC antigen expression at the site of inflammation. Comparable to its strong stimulation of MHC antigen expression in vitro, the intravenous injection of recombinant interferon-γ into mice causes a widespread induction and increase of both class-I and class-II antigens [27].

In the absence of stimulatory factors, cells in culture can spontaneously lose class-I antigens [28]. In addition, several substances can actively suppress the MHC antigen expression in vitro and in vivo. Prostaglandins, α-fetoprotein, and corticosteroids inhibit class-II antigen expression on mouse macrophages even in the presence of interferon-γ [29–31]. In vivo, local suppression of class-II antigen expression was observed when prednisolone was infused directly into rat renal allografts [32]. The administration of cyclosporine to dogs suppresses the expression of class-II antigens on endothelial cells that normally are positive for these antigens [33].

We have tried to relate the adaptation seen in murine skin grafts to changes in expression of MHC antigens. The presence of MHC antigens in the grafts was investigated by in-vivo injection of radiolabeled anti-class-I and anti-class-II monoclonal antibodies, which were specifically directed against the MHC antigens of the donor strain [34, 35]. Four hours after the injection, the grafts were excised and the amount of radioactivity of the entire graft was determined by γ-counting. By using autoradiography, and in additional studies with fluorescent antibodies, we could demonstrate that the antibody had become bound to the capillary endothelium of the graft. Figure 8 shows the changes in the binding capacity of skin allografts for a monoclonal antibody against class-I antigen on different days after transplantation. Cellular rejection of these grafts was prevented by daily administration of cyclosporine to the recipients. We observed maximal binding (that is, maximal expression of MHC antigens) at days 5 and 8 after transplantation; binding ability thus correlated with the period of maximal vulnerability to antibody-mediated destruction. After day 10, binding of antibody gradually decreased to a background level, which was reached at day 18. Skin grafts that were MHC-compatible with the recipient strain and that were carried by the same recipients show a background uptake of radiolabeled antibody throughout the experiment. We observed comparable

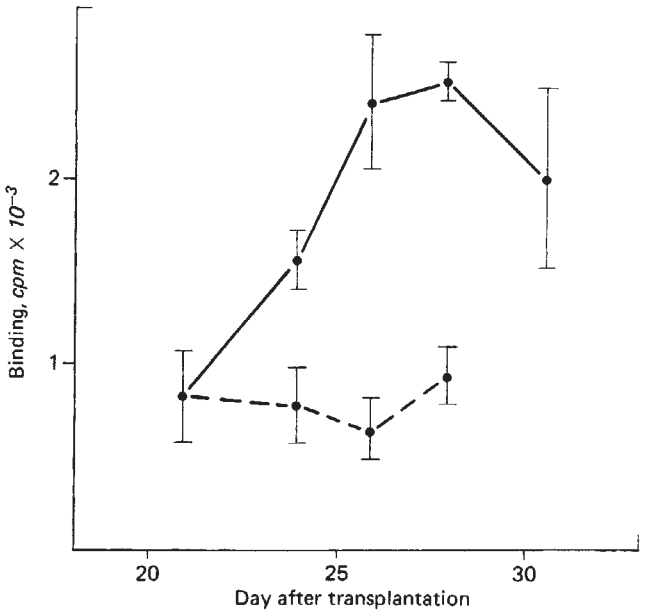

Fig. 9. *Influence of immunosuppressive treatment on the in-vivo binding of radiolabeled anti-class-I monoclonal antibody to mouse skin allografts.* The recipients were treated daily with cyclosporine, for 3 weeks. Treatment was discontinued on day 21 after transplantation in one group (●——●), whereas the control group (●---●) received cyclosporine throughout. (From Ref. 35.)

changes when we used specific antibodies against class-II antigens [34].

The occurrence of a gradual decrease of MHC antigen expression after transplantation would elegantly explain the adaptation of longstanding murine skin grafts. Decreased expression is maintained provided that the immune response of the host remains suppressed. This adaptation is illustrated by the experiment depicted in Figure 9. When cyclosporine treatment was withdrawn at day 21 after transplantation, we detected enhanced class-I antibody binding as compared with that in a control group receiving cyclosporine throughout the experiment. Again, comparable observations were made with anti-class-II antibodies [34]. A low expression of MHC antigens in longstanding grafts probably represents a quiescent state typical of normal, healthy tissue and is a state regained by a graft after a period of increased expression induced by the transplantation procedure and the accompanying inflammatory processes. Milton and coworkers demonstrated that not only antigenic stimuli but also the trauma of the transplantation operation itself causes an increased expression of MHC antigens [36]. Isografts of hearts and kidneys in rats showed a transiently increased expression of class-I antigens immediately after transplantation, even though the hosts could not mount an allogeneic immune response against such grafts. The fact that longstanding skin grafts again become sensitive to antibody-mediated rejection after retransplantation onto new hosts also could be explained by an increase in antigen expression.

What is the evidence that an increased expression of MHC antigens makes a graft more sensitive to rejection? In-vitro studies have established that the extent of lysis of target cells induced by MHC antibodies correlates with the concentration of the target antigen on the cell membrane [37]. No direct evidence indicates that this correlation also holds for the destruction by cytotoxic T-cells. But arguments that cytotoxic T-cell activity correlates with the amount of antigen on the cell membrane can be derived from the observation that increased expression of class-I antigens on tumor cells makes these cells more sensitive to MHC-restricted cell-mediated lysis [38]. A relationship between antigen concentration and damaging potential of the corresponding MHC antibodies also has been demonstrated in vivo in animal studies in which transplanted allogeneic tumors were used as target tissues [39].

The experimental data are fairly solid, but it is difficult to obtain direct proof that the variability of MHC antigen expression plays a role in long-term graft acceptance in clinical renal transplantation. Several studies demonstrate that class-I and class-II antigen expression increase in the kidney during rejection episodes [40, 41]. The increase in class-II expression on renal tubular cells even can be used as a diagnostic sign of rejection [41]. It remains difficult, however, to determine whether this increase in class-II expression plays an active role in the rejection process or whether this rise is merely a consequence of rejection. Some observations suggest that the increased expression is not simply a bystander phenomenon. Treatment of renal transplant patients with recombinant α-interferon to prevent viral infection has led to severe rejection episodes [42]. As I mentioned before, interferons are the strongest inducers of MHC antigens. Viral infections lead to an increase of systemic interferon levels [43] and often are associated with renal graft rejection [44, 45]. An increase in MHC antigen expression might be the triggering mechanism in these rejections. It is possible that the immunosuppressive effects of cyclosporine and corticosteroids are at least partly related to their suppressive effects on the expression of MHC antigens; the studies I mentioned earlier bolster this possibility [31–35]. A low level of MHC antigen expression also might explain why liver grafts are less susceptible to rejection than are grafts of other organs [46].

Decreased vascularization. The classic histologic studies by Medawar of skin grafts in rabbits showed that a rich vascular network is formed during ingrowth of the grafts [47]. The vessels decrease in size after day 8 and are comparable to normal skin vessels around day 24. In mice, similar changes in the vascularization of skin autografts have been described [48, 49]. It is conceivable that the degree of vascularization greatly determines the accessibility of the graft antigens to antibody or to cytotoxic lymphocytes. The disappearance of hypervascularization in skin grafts coincides with the decrease of sensitivity to antibody-mediated rejection and thus might be responsible for the adaptation of skin grafts. In this concept, adaptation is not an actual decrease in sensitivity of the grafted tissue itself. Rather, adaptation merely reflects the recovery of the graft from the trauma of the transplantation procedure, which caused a transient state of hypervascularization. A decreased degree of vascularization as a cause of adaptation perfectly fits with the observations in the clinical case that we are discussing today.

The role of hemodynamic factors in immunologically mediated diseases

Although the main topic of this forum is adaptation of an allograft to immunologic attack by the host, the protection of

part of the graft in the patient we are discussing leads us to examine other clinical situations in which an immune disease is influenced by circulatory effects. In this regard the patient under discussion shows many similarities to the rare patient with unilateral glomerulonephritis; only 9 such patients were reported between 1944 and 1985. In all 9, blood flow to the kidney that was protected from the injury was decreased. Two of the 9 had a hypoplastic kidney as a consequence of long-standing hydronephrosis [50, 51]; all the others had a renal artery stenosis [52–55]. The causes of the glomerulonephritis were variable. The first description of unilateral glomerulonephritis by Fahr in 1944 concerned a patient with diffuse extracapillary glomerulonephritis and a rapidly progressive clinical course [50]. A second patient with this disease was reported 30 years later [51]. Four of the 9 patients had proliferative glomerulonephritis [51–54], and 2 had glomerulonephritis secondary to systemic disease; one of these had lupus nephritis [54] and the other patient was a child with Schönlein-Henoch purpura [51]. The ninth patient is especially interesting, because it is the only case in which immunofluorescence and electron-microscopic studies of both the ischemic and the nonischemic kidney were performed [55]. Nephrotic syndrome developed during treatment with captopril for severe hypertension. Arteriography revealed that the right renal artery, which had been patent before treatment with captopril, had become occluded and appeared to be perfused only by collateral vessels. The left kidney (which had a patent artery) showed typical signs of membranous glomerulonephritis. Immunofluorescent studies revealed extensive granular deposits of IgG, IgM, and C3 in the glomerular capillary wall. Electron microscopy disclosed large, electron-dense deposits on the subepithelial side of the glomerular basement membrane, findings characteristic of membranous glomerulonephritis. In the right (ischemic) kidney, the glomeruli manifested only ischemic changes, and immunofluorescent examination was negative. Some sparsely distributed, very small deposits were visible at the epithelial side of the glomerular basement membrane on electron microscopy. This latter finding suggests not that the deposition of immunoglobulins was prevented by decreased perfusion, but rather that their further aggregation to larger immune complexes was inhibited.

The influence of local perfusion on the induction of immunologic damage also is apparent from the observations that, in biopsies of patients with acute glomerulonephritis, ischemic glomeruli are not affected [56, 57]. This observation raises the question of whether intrarenal differences in perfusion also are responsible for the focal and segmental character of some immunologically mediated forms of glomerulonephritis, such as systemic lupus erythematosus or Schölein-Henoch purpura.

In experimental studies, only a few investigators have focused on local factors that might determine the localization of vascular lesions. Artificially induced hypertension aggravated the glomerular, cardiac, and vascular lesions in rabbits with acute serum sickness [58]. In a similar model, the inflammatory lesions in large arteries were most prominent at areas of high turbulence [59]. The induction of clip hypertension in rats with nephrotoxic serum nephritis also increased glomerular proliferation and sclerosis in the non-clipped kidney [60]. On the other hand, Germuth and colleagues more than 20 years ago showed that the acute, proliferative, and exudative lesions in rabbits with acute serum sickness were reduced, or even completely prevented, if the renal artery was narrowed by 50% or if the ureter was ligated. Moreover, immunofluorescent examination revealed no deposition of IgG in the unaffected glomeruli, whereas extensive histologic lesions and immune deposits were apparent in the contralateral kidney [61]. These results suggest that hemodynamic factors play an important role in inducing immune-mediated damage. A more recent study tried to characterize these factors more specifically [62]. Hebert et al measured the uptake of infused radiolabeled immune complexes in kidneys of normal dogs in which the blood flow to one kidney was decreased by renal artery constriction or by elevation of the ureteral pressure. They found a linear relationship between immune-complex uptake and the reduction in renal blood flow. They reasoned that capillary hydrostatic pressure itself could not be the most important factor in the trapping of immune complexes, because both causes of decreased renal blood flow (that is, renal artery stenosis and elevation of ureteral pressure) gave similar results, although they have opposite effects on capillary hydrostatic pressure. It remains doubtful whether these findings are fully representative of the events occurring during glomerulonephritis, because the vast majority of the infused immune complexes appeared to be trapped at nonglomerular sites in Hebert's experiments. The significance of these findings also is questionable in light of the new findings on the causes of glomerulonephritis [63]. It appears that the binding of antibodies in the glomerulus to fixed or planted antigens plays a much more important role than does deposition of circulating immune complexes. Thus, although it is obvious that decreases in perfusion can protect the kidney against immunologic damage, the exact mechanism of this protection remains unclear.

Renal artery stenosis also can protect a kidney against damage that is presumably nonimmunologic. The classic examples are protection against the direct damaging effects of high blood pressure in patients with hypertension and unilateral renal artery stenosis, and protection of animals with experimental Goldblatt hypertension [64]. It is difficult to understand how unilateral renal artery stenosis can protect a kidney against diabetic nodular glomerulosclerosis, however [65]. This unique observation conforms with the earlier notion that the nodular lesions of diabetic nephropathy do not develop in ischemic glomeruli [56]. Indeed, these observations support the recent view that glomerular hyperfiltration, probably in combination with raised blood pressure, is an important determinant of diabetic nephropathy [66].

Summary

The experimental and clinical data suggest that both a decrease of antigen expression and decreased perfusion can protect against immunologically mediated destructive processes. In the adaptation of skin grafts, these factors could be interrelated. Inadequate perfusion might lead to a decreased delivery of substances that stimulate MHC antigen expression. This course of events also could explain the protection in the patient presented here. Immune deposits were completely absent in the protected segment of the kidney, although immune deposits were abundantly present in the remaining part of the kidney, and circulating anti-donor antibodies were demonstrable after the transplanted kidney had been removed. The limited

availability of frozen biopsy material has prevented us from comparing the expression of MHC antigens in both kidney segments using monoclonal antibodies. But such studies might be done in experimental kidney transplants with an artificially induced stenosis of the renal artery.

Except when an arterial stenosis is present, we have little reason to assume that perfusion gradually decreases in longstanding kidney grafts as it does in skin grafts. Therefore, if adaptation plays a role in the gradual decrease of the sensitivity to rejection in longstanding kidney grafts, this phenomenon must be attributed to a decreased expression of target antigens as a consequence of factors other than decreased perfusion. The most likely candidates are the immunosuppressive drugs, such as cyclosporine and prednisone, which decrease MHC antigen expression.

Let me conclude by returning to my main theme of graft adaptation. It seems appropriate to end this review with a quotation from one of Woodruff's original publications on this subject: "If the phenomenon [adaptation] applies to homotransplants of normal tissues to sites other than the eye, I think it almost certain that the clinical homograft problem will be solved; if it does not, the problem may prove insoluble" [9]. Although our insight into the rejection process has increased considerably, we still do not know which factors are most important in determining the long-term survival of primarily vascularized grafts.

Questions and answers

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): Could you comment on the possible interplay between host and graft factors involved in acceptance of the graft? For instance, is there any link between down-regulation of the expression of MHC antigens and deletion of antigen-specific cells?

PROF. KOENE: This is a very complicated problem. I would like to mention one interesting observation that stems from the study in which prednisolone was infused locally into rat kidney grafts [32]. These grafts showed a decrease in class-II antigen expression and had a prolonged survival, whereas the number of lymphocytic infiltrates present in these kidneys was the same as those in untreated control grafts. So the presumed effector lymphocytes were there, but apparently they were not stimulated to set off the chain of events that leads to rejection. This observation shows that down-regulation of MHC antigen expression does not necessarily lead to elimination of antigen-reactive effector cells. These observations also explain why we often find lymphocytic infiltrates in the renal biopsies of patients treated with cyclosporine, or with azathioprine and prednisone, while there are no clinical signs of rejection. I think that it is more important to look for large, activated lymphocytes. These are not easy to identify in core biopsies, but they can be seen in fine-needle aspirates during acute rejection episodes, as shown by Bishop and coworkers [67].

DR. JORDAN J. COHEN (*Dean of Medicine, State University of New York at Stony Brook, Stony Brook, New York*): What is the relationship between "adaptation" and graft "tolerance?"

PROF. KOENE: In my opinion these should be considered two essentially different phenomena. As I briefly mentioned before, the term adaptation has been used by some authors in a broader sense to include a decreased immunogenicity of the graft by the disappearance of passenger cells from the graft. In the definition that I have adopted, which originally was proposed by Woodruff [9], adaptation is the protection of a graft from an already established immune response. In a similarly confusing way, the term tolerance has been used to describe completely different mechanisms of graft acceptance. It is often used as a broad term, meaning that the graft is tolerated by the host by any possible protective mechanism; but in the strict sense, tolerance refers to removal or inactivation of specific antigen-reactive cells from the host. If both terms are used in their strict sense, the most important difference between adaptation and tolerance is that in adaptation the immune response against the graft is still active, whereas in tolerance this response is absent or completely suppressed.

PROF. PEKKA HÄYRY (*Professor of Transplantation Surgery and Immunology, University of Helsinki, Helsinki, Finland*): I believe that in your "adaptation" mechanism, several systems are operating together. For example, if inflammation is induced in a part of the graft, it might protect another part of the graft in the same way as a large liver graft can protect a kidney or a heart graft. This might be one explanation for only part of the kidney being rejected in the patient under discussion. Second, I am not sure whether endothelium is replaced in many of the grafts. In primarily vascularized grafts, it is not. In skin it must be, and in pancreatic islets, donor capillary endothelium is replaced by recipient endothelium. Would you please comment on these possibilities?

PROF. KOENE: Your first remark relates to the fact that a large graft might keep the effector systems so busy that another grafted tissue might escape from rejection. Indeed, one can imagine that in cell-mediated rejection, there might not be enough cells available to do the work somewhere else. Experimental evidence shows that this can occur, but it is more difficult to conceive that the same will happen when circulating antibody is present. The patient I described had a chronic vascular rejection most likely mediated by circulating antibodies, as can be deduced from the histology of the graft and from the demonstration of cytotoxic antibodies in the serum. I find it difficult to understand why the antibody does not deposit in the graft when one can see contrast medium reaching the poststenotic part of the kidney and when antibody is present in measurable titers in the circulation. Repair mechanisms could be obscuring destructive changes, but these still would not explain why immunofluorescence was negative in this part of the graft.

Your second question implies that you believe that skin grafts show replacement of endothelium. I do not think that endothelial replacement takes place in the skin. We have been able to show persistent expression of donor antigens on the vascular endothelium of rat skin xenografts even after 170 days of residence [22]. Pancreatic islet grafts are different in this regard, because such grafts represent a nonvascularized tissue that will be invaded by host vessels after transplantation; this occurrence is similar to the invasion of a transplanted tumor by host vessels.

PROF. HÄYRY: Are you certain that chronic vascular rejection is mediated by antibody and not by cells?

PROF. KOENE: There is convincing experimental and clinical evidence that antibodies, if administered or if present in the host, can damage the graft.

PROF. HÄYRY: But that is only the case in hyperacute rejection. There are very few papers on the role of antibody in chronic rejection.

PROF. KOENE: True, but if chronic vascular rejection had an important cellular component, one would expect to find a histologic correlate thereof. We do not see lymphoid effector cells in the vessels, however. We only see destruction of endothelial cells, platelet aggregation, intravascular coagulation, and intimal fibrosis.

PROF. LEENDERT A. VAN ES (*Chief, Department of Nephrology, University of Leiden, Leiden, The Netherlands*): My question pertains to delivery versus concentration of mediators. We can reasonably assume that decreased flow will lead to decreased delivery of immunocompetent cells or antibodies when these are present in limited amounts. This is more difficult to understand if an excess of antibody is present. If the concentration rather than flow of lymphokines or other mediators is important, I would expect that a renal artery stenosis would play less of a role. Can you explain the protection of the lower part of the graft fully by hemodynamic factors?

DR. KOENE: In the situation that we are talking about, there is probably continuous stimulation of the immune response, and the antibody is chronically delivered. I fully agree with you that it is difficult to understand why antibody is not bound, even in a poorly perfused graft, if it is continuously present in the circulation. Therefore I am inclined to believe that the disappearance of MHC antigens from the vascular endothelium protected the poorly perfused segment.

PROF. YVES PIRSON (*Renal Physician, Department of Nephrology, Cliniques Universitaires St. Luc, Brussels, Belgium*): In a series of animal experiments, Monaco demonstrated increased tolerance of kidney grafts by infusing donor bone marrow a few days after renal transplantation [68]. He was able to show the establishment of donor cells (possibly suppressor cells) in some grafts. Do you think that such a procedure could be applied in clinical transplantation?

PROF. KOENE: The experiments you are referring to were performed in mice that received allogeneic skin grafts followed by an injection of donor bone marrow 6 days later [68]. This model is complex, because the recipient mice also were treated with antilymphocyte serum. The donor cells responsible for the graft prolongation have not been precisely identified, but evidence suggests that they possess suppressive activity in mixed lymphocyte reactions in vitro. Ildstad and Sachs made another fascinating observation at the U.S. National Institutes of Health. They found that the reconstitution of bone marrow in irradiated mice with a mixture containing both T-cell-depleted syngeneic and allogeneic or xenogeneic bone marrow cells leads to specific acceptance of skin allografts or xenografts [69]. It will be necessary to show that these manipulations are also effective in larger animals, but if so, this certainly seems an attractive approach for obtaining specific graft tolerance in clinical transplantation.

PROF. FRITZ BÜHLER (*Head, Hypertension Unit, University Hospital, Basel, Switzerland*): Cyclosporine suppresses class-I and class-II antigen expression, and it is strongly immunosuppressive. Why are aspirin and other nonsteroidal antiinflammatory drugs, which interfere with prostaglandin synthesis, not immunosuppressive?

PROF. KOENE: The prostaglandin antagonists would be expected to decrease, and not increase, graft survival, because prostaglandins suppress MHC antigen expression. Theoretically, the inhibitors would cause an increase of MHC antigen expression and make the graft more sensitive to rejection. Most studies have confirmed that these agents heighten the immune response in vitro and in vivo [70]. With regard to graft rejection, the results are controversial. Some workers have found prolonged skin-graft survival in the mouse when indomethacin was given along with low doses of corticosteroids [71]. Others, however, have found decreased survival when indomethacin was used alone [72].

PROF. BÜHLER: But is the adaptation process the key event in long-term graft acceptance?

PROF. KOENE: I do not believe that there is one single key mechanism. What we observe is that the sensitivity of a graft to the rejection process decreases with time. The longer the graft is there, the less sensitive it will be. As I showed you, different factors can play a role in long-term graft acceptance. In the complex clinical situation, it is very difficult to determine the precise role of each factor. The important thing I want to point out is that adaptation is very likely to be one of these factors. It is a mechanism that has not received much attention in the past. The study of its role might teach us how we can control rejection even better than we do now. We do quite a good job by reaching one-year survival rates between 80% and 90% in renal allografts, but still these results are obtained at the expense of potentially dangerous immunosuppressive therapy.

PROF. JAN J. WEENING (*Chief, Division of Immunopathology, University of Groningen, Groningen, The Netherlands*): What were the effects of IL-2 treatment late, and of anti-class-II antibodies early, in the murine transplantation model?

PROF. KOENE: You raise two different problems. We have not tried IL-2 treatment in our mouse model, but we have tried to increase MHC antigen expression and to induce antibody-mediated rejection in longstanding grafts by treatment with γ-interferon. So far we have not been successful. We are, however, not certain that enough of the systemically administered interferon was delivered to the graft. We have not measured that. We currently are trying to increase MHC expression by the administration of mixed lymphocyte culture (MLC) supernatants and also by the transfer of sensitized lymphocytes, but results of these experiments are not yet available.

Your second question relates to the effects of administration of anti-class-II antibody early after transplantation. Antibodies given at transplantation or within the first days thereafter can have two opposite effects: they can be destructive if they bind to the graft and activate secondary mediators, such as the complement system. However, if they cannot destroy the graft by themselves (as is the case for alloantibodies in the mouse because they activate complement very poorly), they paradoxically can block the immune response. That is an example of so-called passive immunologic enhancement. It is probably a central blockade of the immune response in which the antibody, together with antigen released from the graft, becomes bound as an immune complex to the antigen-reactive cells. These cells are then opsonized and the immune response is delayed or even completely suppressed [73].

PROF. HÄYRY: What about noncompliant patients? Do the

patients who stop taking immunosuppressive drugs reject their grafts?

PROF. KOENE: We all know patients whom we suspected of not taking their drugs and who rejected their grafts. Also, patients with chronic hepatitis who could not be given high enough doses of azathioprine have rejected their grafts even 5 or 6 years after transplantation. On the other hand, there are isolated reports of patients who tolerated their graft even though they stopped taking immunosuppressive drugs some years after transplantation. Certainly some patients can do without any immunosuppression once their grafts have become established, but these individuals seem to be the exception to the rule, and we have no means of identifying them. The only way to do this is by discontinuing treatment and waiting for a rejection to occur. Obviously, this is not an advisable approach.

DR. HARRINGTON: I believe I can answer in part Dr. Häyry's question on noncompliance in transplant patients. Several years ago we collected some interesting data on what happens to transplant patients who stop taking their immunosuppressive medications [74]. We identified 48 patients in the U.S. who had stopped therapy on their own from a total transplant population of over 6000. Of the 48 patients, 21 experienced graft failure before the noncompliance was discovered, and only 6 did well for a prolonged period. Our data demonstrated that at no point after transplantation is it safe to discontinue immunosuppressive therapy. The data also demonstrate that noncompliance in transplant patients is exceedingly unusual.

DR. EMILE DE HEER (*Department of Pathology, University of Leiden, Leiden, The Netherlands*): You probably are familiar with the kidney transplantation model described by Hutchinson and Morris, in which they induce graft acceptance by donor-specific blood transfusions [75]. Cultured infiltrating T-cells from tolerant rats turned out to be graft-specific cytotoxic T-cells that had failed to exert their cytotoxic function in vivo. Do you think that this failure in their model is caused by decreased expression of target epitopes in the graft as a consequence of adaptation?

PROF. KOENE: That seems an attractive explanation. The graft prolongation in the model you mention is most probably caused by T-suppressor cells [75]. It is conceivable that suppression of the immune response by these cells prevents the production of lymphokines and thereby MHC antigen expression, so that targets for the cytotoxic cells on the endothelium of the graft are absent. One should not forget, however, that many different immunosuppressive mechanisms play a role in clinical transplantation. Goulmy et al showed that patients with longstanding and well-functioning grafts can lose cell-mediated lympholysis reactivity against their donors [1]. This loss might be due to the appearance of suppressor cells or to the disappearance of antigen-reactive cells. The non-reactivity was specific for the HL-A antigens of the donor. Alternatively, anti-idiotypic antibodies can develop that cause specific blockade [2]. Graft adaptation may be another factor that protects longstanding grafts against rejection. It seems likely that none of these protective mechanisms will by itself guarantee long-term graft survival, but rather that a combination of mechanisms and their interplay ultimately are responsible for protection of the graft.

DR. DE HEER: Was the failure of the effector cells in exerting their function in the in-vitro experiments that you mentioned due to the disappearance of MHC antigens from the target cells?

PROF. KOENE: Not in the studies by Goulmy et al. This group used donor lymphoid cells that were collected at the time of grafting.

PROF. PIERRE VERROUST (*Director of Research, INSERM, Hôpital Tenon, Paris, France*): What is the mechanism of protection of thyroid grafts in the eye in the experiments by Woodruff?

PROF. KOENE: Woodruff hypothesized that the anterior chamber of the eye is an immunologically privileged site and that this exemption is related to the absence of vascularization [8]. One explanation is that the graft becomes vascularized so slowly that it already has undergone adaptive changes and has become resistant to the rejection mechanisms. It seems more likely, however, that the location in the anterior chamber decreases the immunogenicity of the graft because the passenger cells that have to present the antigen cannot leave the anterior chamber and thus cannot reach the lymph nodes. After vascularization has become established, these cells already have disappeared by cell death; this is similar to what happens when thyroid tissue is cultured during a few days in vitro. The lymphoid cells in the tissue do not survive this procedure. Subsequent grafting of the cultured thyroid does not lead to a rejection response [4, 5]. This finding also explains why a concomitantly placed subcutaneous graft, which contains many passenger cells and is strongly immunogenic, will destroy the graft in the eye.

PROF. HÄYRY: I wish to offer a word of warning about the different tolerance models. These models might look the same superficially, but they are different. The extent of inflammation in the kidney is vastly different. In cyclosporine A-induced tolerance in the rat, there is very little inflammation. In anti-thymocyte-globulin-induced tolerance, there is much inflammation, and this is also the case in "tolerance" induced by donor-specific transfusions (DST) in the rat. Do you agree?

PROF. KOENE: I completely agree that the mechanisms of so-called tolerance in different models vary widely. But I do not agree with your explanation of the mechanism of the beneficial effect of DST. It is not at all certain that the DST by itself leads to specific immunosuppression. It is important to realize that in clinical transplantation, recipients of living-donor kidneys treated with donor-specific transfusions before transplantation can develop severe rejection episodes very early after transplantation. Fortunately, these rejections respond very well to anti-rejection treatment, and thereafter the graft generally is tolerated without further problems. This somewhat paradoxical course in these patients has led to the speculation that DST actually might immunize the recipient to the donor antigens [76]. The ultimately favorable course would be explained by the fact that in the severe rejection that occurs early after transplantation, the majority of cells reactive to the donor antigens will participate. These cells are then effectively eliminated by the rejection treatment, so that ultimately a state of tolerance develops, that is, the absence of donor-specific antigen-reactive cells. Whether this explanation of the beneficial effect of DST is correct remains to be seen, but it can be concluded that the inflammatory changes seen initially in the graft after DST could reflect rejection activity and not tolerance.

Acknowledgments

The author is grateful to Dr. M. J. J. T. Bogman for help and advice in the preparation of the manuscript. He wishes to thank Drs. K. J. M. Assmann, W. J. M. Tax, J. F. M. Wetzels, and J. G. J. van de Winkel for critically reading the manuscript.

Reprint requests to Prof. R. A. P. Koene, Department of Medicine, Division of Nephrology, University of Nijmegen, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands

References

1. Goulmy E, Blokland E, Persijn G, Paul LC, Wilmink J, van Rood J: HLA regulates postrenal transplant CML nonreactivity. *J Immunol* 135:3028–3086, 1985
2. Reed E, Hardy M, Benvenisty A, Lattes C, Brensilver J, McCabe R, Reemtsma K, King DW, Succiu-Foca N: Effect of antiidiotypic antibodies to HLA on graft survival in renal-allograft patients. *N Engl J Med* 316:1450–1455, 1987
3. Bellanti JA: Immunologically mediated disease, in *Immunology III* (3rd ed), edited by Bellanti JA, Tokyo, Saunders, 1985, pp 346–446
4. Lafferty KJ, Cooley MA, Woolnough J, Walker KZ: Thyroid allograft immunogenicity is reduced after a period in organ culture. *Science* 188:259–261, 1975
5. Lacy PE, Davie JM, Finke EH: Prolongation of islet allograft survival following in vitro culture (24°C) and a single injection of ALS. *Science* 204:312–313, 1979
6. Talmage C, Dart G, Radovich J, Lafferty K: Activation of transplant immunity: effect of donor leukocytes on thyroid allograft rejection. *Science* 191:385–388, 1976
7. Lacy PE, David JM, Finke EH: Induction of rejection of successful allografts of rat islets by donor peritoneal exudate cells. *Transplantation* 28:415–420, 1979
8. Woodruff MFA, Woodruff HG: The transplantation of normal tissues: with special reference to auto- and homotransplants of thyroid and spleen in the anterior chamber of the eye, and subcutaneously, in guinea-pigs. *Philosoph Trans R Soc Lond (Biol)* 234:559–581, 1950
9. Woodruff MFA: The "critical period" of homografts. *Transpl Bull* 1:221–222, 1953
10. Woodruff MFA, Simpson LO: Induction of tolerance to skin homografts in rats by injection of cells from the prospective donor soon after birth. *Br J Exp Pathol* 36:494–499, 1955
11. Nirmul GO, Severin C, Taub RN: Adaptation of skin allografts in mice treated with antilymphocyte serum. *Transplantation* 13:27–30, 1972
12. McKenzie IFC, Koene RAP, Winn HJ: Evidence for adaptation of skin grafts in enhanced, irradiated mice. *Transplantation* 14:661–663, 1972
13. Stuart FP, Fitch FW, Rowley DA: Specific suppression of renal allograft rejection by treatment with antigen and antibody. *Transpl Proc* 2:483–484, 1970
14. Fabre JW, Morris PJ: The mechanism of specific immunosuppression of renal allograft rejection by donor strain blood. *Transplantation* 14:634–640, 1972
15. Lechler RJ, Batchelor JR: Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. *J Exp Med* 155:31–41, 1982
16. Koene RAP, Gerlag PGG, Hagemann JHFM, van Haelst UJG, Wijdeveld PGAB: Hyperacute rejection of skin allografts in the mouse by the administration of alloantibody and complement. *J Immunol* 111:520–526, 1973
17. Baldamus CA, McKenzie IFC, Winn HJ, Russell PS: Acute destruction by humoral antibody of rat skin grafted to mice. *J Immunol* 110:1532–1541, 1973
18. Gerlag PGG, Capel PJA, Hagemann JFHM, Koene RAP: Adaptation of skin grafts in the mouse to antibody-mediated rejection. *J Immunol* 125:583–586, 1980
19. Gerlag PGG, Koene RAP, Hagemann JFHM, Wijdeveld PGAB: Hyperacute rejection of skin allografts in the mouse. Sensitivity of ingrowing skin grafts to the action of alloantibody and complement. *Transplantation* 20:308–313, 1975
20. Dvorak HF, Mihm MC, Dvorak AM, Barnes BA, Manseau EJ, Galli SJ: Rejection of first-set skin allografts in man. The microvasculature is the critical target of the immune response. *J Exp Med* 150:322–337, 1979
21. Jooste SV, Colvin RB, Winn HJ: The vascular bed as the primary target in the destruction of skin grafts by antiserum. II. Loss of sensitivity to antiserum in long-term xenografts of skin. *J Exp Med* 154:1332–1341, 1981
22. Bogman MJJT, de Waal RMW, Koene RAP: Persistent expression of donor antigens in endothelium of long-standing skin xenografts and vulnerability to destruction by specific antibodies. *Transpl Proc* 29:205–207, 1987
23. Hart DNJ, Winearls CG, Fabre JW: Graft adaptation: Studies on possible mechanisms in long-term surviving renal allografts. *Transplantation* 30:73–80, 1980
24. Burdick JF, Russell PS, Winn HJ: Sensitivity of long-standing xenografts of rat hearts to humoral antibodies. *J Immunol* 123: 1732–1735, 1979
25. Koene RAP, de Waal RMW, Bogman MJJT: Variable expression of major histocompatibility antigens: Role in transplantation immunology. *Kidney Int* 30:1–8, 1986
26. Halloran PF, Wadgymar A, Autenried P: The regulation of expression of major histocompatibility products. *Transplantation* 41:413–420, 1986
27. Skoskiewicz MJ, Colvin RB, Schneeberger EE, Russell PS: Widespread and selective induction of major histocompatibility complex-determined antigens in vivo by γ-interferon. *J Exp Med* 162:1645–1664, 1985
28. Ball ED, Guyre PM, Glynn JM, Rigby WFC, Fanger MW: Modulation of Class I HLA antigens on HL-60 promyelocytic leukemia cells by serum-free medium: re-induction by γ-IFN and 1,25-dihydroxyvitamin D_3 (calcitriol). *J Immunol* 132:2424–2429, 1984
29. Snyder DS, Beller DI, Unanue ER: Prostaglandins modulate macrophage expression of Ia antigens. *Nature* 299:163–165, 1982
30. Lu CY, Changelian PS, Unanue ER: α-fetoprotein inhibits macrophage expression of Ia antigen. *J Immunol* 132:1722–1727, 1984
31. Snyder DS, Unanue ER: Corticosteroids inhibit murine macrophage Ia expression and interleukin 1 production. *J Immunol* 129:1803–1805, 1982
32. Ruers TJM, Buurman WA, van Boxtel CJ, van der Linden CJ, Kootstra G: Immunohistological observations in rat kidney allografts after local steroid administration. *J Exp Med* 166:1205–1220, 1987
33. Groenewegen G, Buurman WA, van der Linden CJ: Lymphokine dependence of in vivo expression of MHC class II antigens by endothelium. *Nature* 316:361–363, 1985
34. De Waal RMW, Bogman MJJ, Maass CN, Cornelissen IMHA, Tax WJM, Koene RAP: Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. *Nature* 303:426–429, 1983
35. De Waal RMW, Bogman MJJT, Cornelissen IMHA, Vermeulen AN, Koene RAP: Expression of donor class I major histocompatibility antigens on the vascular endothelium of mouse skin allografts. *Transplantation* 42:178–183, 1986
36. Milton AD, Spencer SC, Fabre JW: The effects of cyclosporine on the induction of donor class I and class II antigens in heart and kidney allografts in the rat. *Transplantation* 42:337–347, 1986
37. Möller E, Möller G: Quantitative studies of the sensitivity of normal and neoplastic mouse cells to the cytotoxic action of isoantibodies. *J Exp Med* 115:527–553, 1962
38. Doherty PC, Knowles BB, Wettstein PJ: Immunological surveillance of tumors in the context of major histocompatibility complex restriction of T cell function. *Adv Canc Res* 42:1–65, 1984
39. Winn HJ: Immune mechanisms in homotransplantation. I. The role of serum antibody and complement in iso-immune reactions. *J Immunol* 84:530–538, 1960
40. Häyry P, von Willebrand E, Ahonen J, Eklund B: Do well-to-do and repeatedly rejecting renal allografts express the

transplantation antigens similarly on their surface? *Scand J Urol Neprhol* 64:52–55, 1981
41. HALL BM, BISHOP GA, DUGGIN GG, HORVATH JS, PHILIPS J, TILLER DJ: Increased expression of HLA-DR antigens on renal tubular cells in renal transplants: Relevance to the rejection response. *Lancet* 2:247–251, 1984
42. KRAMER P, BIJNEN AB, TEN KATE FWJ, JEEKEL J, WEIMAR W: Recombinant leucocyte interferon A induces steroid-resistant acute vascular rejection episodes in renal transplant recipients. *Lancet* 1:989–990, 1984
43. WHEELOCK EF, SIBLEY WA: Interferon in human serum during clinical viral infections. *Lancet* 2:382–385, 1964
44. MAY AG, BETTS RF, FREEMAN RB, ANDRUS CH: An analysis of cytomegalovirus infection and HLA antigen matching on the outcome of renal transplantation. *Ann Surg* 187:110–117, 1978
45. MARKER SC, HOWARD RJ, SIMMONS RL, KALIS JM, CONNELLY DP, NAJARIAN JS, BALFOUR HH: Cytomegalovirus infection: a quantitative prospective study of three hundred twenty consecutive renal transplants. *Surgery* 89:660–671, 1981
46. CALNE RY, MCMASTER P, PORTMANN B, WALL WJ, WILLIAMS R: Observations on preservation, bile drainage and rejection in 64 human orthotopic liver allografts. *Ann Surg* 186:282–290, 1977
47. MEDAWAR PB: The behaviour and fate of skin autografts and skin homografts in rabbits. *J Anat* 78:176–199, 1944
48. CASTERMANS A: Vascularization of skin grafts. *Transplant Bull* 4:153–154, 1957
49. ROLLE GK, TAYLOR AC, CHARIPPER HA: A study of vascular changes in skin grafts in mice and their relationship to homograft breakdown. *J Cell Comp Physiol* 53:215–239, 1959
50. FAHR T: Die Funktionsbehinderung der Niere als hemmendes Moment bei der Entwicklung von Glomerulonephritis und Maligner Nephrosklerose. *Dtsch Arch Klin Med* 191:52–59, 1944
51. DIKMAN SH, STRAUSS L, BERMAN LJ, TAYLOR NS, CHURG J: Unilateral glomerulonephritis. *Arch Pathol Lab Med* 100:480–483, 1976
52. PALMER JM, EVERSOLE SL, STAMEY TA: Unilateral glomerulonephritis. Virtual absence of nephritis in a kidney with partial occlusion of the main renal artery. *Am J Med* 40:816–822, 1966
53. Clinicopathological Conference. A case of aortitis with nephrotic syndrome. *Br Med J* 2:359–365, 1969
54. SALYER WR, SALYER DC: Unilateral glomerulonephritis. *J Pathol* 113:247–253, 1974
55. SMIT AJ, HOORNTJE JJ, WEENING JJ, DONKER AJM, HOEDEMAEKER PJ: Unilateral membranous glomerulopathy during captopril treatment. *Neth J Med* 28:23–27, 1985
56. MCMANUS JFA: Ischemic glomeruli and their significance for glomerular structure. *Am J Pathol* 50:589, 1955
57. HEPTINSTALL RH: Acute glomerulonephritis, in *Pathology of the Kidney* (2nd ed), edited by HEPTINSTALL RH, Boston, Little, Brown, 1974, pp 331–369
58. FISHER ER, BARK J: Effect of hypertension on vascular and other lesions of serum sickness. *Am J Pathol* 39:665–679, 1961
59. KNIKER WT, COCHRANE CG: The localization of circulating immune complexes in experimental serum sickness. The role of vasoactive amines and hydrodynamic forces. *J Exp Med* 127:119–139, 1968
60. NEUGARTEN J, FEINER HD, SCHACHT RG, GALLO GR, BALDWIN DS: Aggravation of experimental glomerulonephritis by superimposed clip hypertension. *Kidney Int* 22:257–263, 1982
61. GERMUTH FG, KELEMAN WA, POLLACK AD: Immune complex disease. II. The role of circulatory dynamics and glomerular filtration in the development of experimental glomerulonephritis. *Johns Hopkins Med J* 120:252–261, 1967
62. HEBERT LA, ALLHISER CL, KOETHE SM: Some hemodynamic determinants of immune complex trapping by the kidney. *Kidney Int* 14:452–465, 1978
63. COUSER WG: What are immune complexes doing in glomerulonephritis? *N Engl J Med* 304:1230–1232, 1981
64. GOLDBLATT H: Experimental renal hypertension. *Am J Med* 4: 100–119, 1948
65. BERKMAN J, RIFKIN H: Unilateral nodular diabetic glomerulosclerosis (Kimmelstiel-Wilson): Report of a case. *Metabolism* 22:715–722, 1973
66. KROLEWSKY AS, CANESSA M, WARRAM JH, LAFFEL LMB, CHRISTLIEB AR, KNOWLER WC, RAND LI: Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *N Engl J Med* 318:140–145, 1988
67. BISHOP GA, HALL BM, WAUGH J, PHILLIPS J, HORVATH JS, DUGGIN GG, JOHNSON JR, SHEIL AGR, TILLER DJ: Diagnosis of renal allograft rejection by analysis of fine-needle aspiration biopsy specimens with immunostains and simple cytology. *Lancet* 2:645–649, 1986
68. DE FAZIO SR, HARTNER WC, MONACO AP, GOZZO JJ: Prolongation of graft survival in ALS-treated mice by donor-specific bone marrow: Density gradient fractionation of the active bone marrow cells. *Transplantation* 43:564–569, 1987
69. ILDSTAD ST, SACHS DH: Reconstitution with syngeneic plus allogenic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature* 307:168–170, 1984
70. GOODWIN JS: Immunologic effects of nonsteroidal anti-inflammatory drugs. *Am J Med* 77(4B):7–15, 1984
71. BELLDEGRUN A, COHEN IC, FRENKEL A, SERVADIO C, ZOR U: Hydrocortisone and inhibitors of prostaglandin synthesis: Potentiation of allograft survival in mice. *Transplantation* 31:407–408, 1981
72. ANDERSON CB, JAFFEE BM, GRAFF RJ: Prolongation of murine skin allografts by prostaglandin E_1. *Transplantation* 23:444–447, 1977
73. LEMS SPM, TAMBOER WPM, CAPEL PJA, KOENE RAP: Effects of IgG and IgM alloantibodies in the enhancement of mouse skin allografts and the relation with their opsonizing capacity in vivo. *J Immunol* 127:665–669, 1981
74. ZOLLER KM, CHO SI, COHEN JJ, HARRINGTON JT: Cessation of immunosuppressive therapy after successful transplantation: A national survey. *Kidney Int* 18:110–114, 1980
75. HUTCHINSON IV, MORRIS PJ: The role of major and minor histocompatibility antigens in active enhancement of rat kidney allograft survival by blood transfusion. *Transplantation* 41:166–170, 1986
76. TERASAKI PI: The beneficial transfusion effect on kidney graft survival attributed to clonal deletion. *Transplantation* 37:119–125, 1984